Evaluation of Water Reclamation for the Tulalip Tribes Quil Ceda Village
Phase I
Professor H. David Stensel
University of Washington
October 23, 2006

Background

An advanced wastewater treatment facility (WWTF) is operated at Quil Ceda Village to process wastewater flows from the Tulalip Casino and surrounding commercial and restaurant establishments. The system uses a state-of-art membrane bioreactor (MBR) with highly efficient solids separation, BOD removal, and nitrogen removal through anoxic/aerobic operating reactors. The final effluent is disinfected by chlorination and is of a much higher quality compared to more common wastewater treatment processes. The system can be expected to produce an effluent with no measurable fecal coliform and suspended solids, total nitrogen less than 5.0 mg/L, and BOD less than 2.0 mg/L. The present permit is for treating a flow of up to 250,000 gallons per day (gpd) and currently the average daily flow is in the range of 120,000 gpd. The treated effluent is presently disposed by subsurface injection using horizontal wells.

The WWTF is expected to handle much higher flows in the future with increased commercial and industrial development on the 2000 acre Quil Ceda Village site. The Everett water settlement on Snohomish River provides up to 40 million gallons per day (MGD) for the Tulalip Tribes Reservation. At some point it may be desirable to discharge treated flow from the WWTF to the nearby Coho Creek, which has flows ranging from 2.0 to 10.0 cubic feet per second (cfs) during the year and with little flow during part of the dry summer months.

One of the major advantages of an MBR system, such as the type installed at the Quil Ceda Village WWTF, is that it produces a Class A effluent in accordance with the State of Washington Department of Ecology (DOE) guidelines, which is of sufficient quality for a wide range of water reuse options. Possible reclaimed water applications at the site include stream augmentation for the Coho Creek, forest and lawn irrigation, and reuse at the WWTF site. According to the State of Washington regulations for reclaimed water use for stream augmentation the discharge must consider downstream uses, meet surface water standards, and comply with the EPA Clean Water Act. In some cases additional requirements are necessary to meet needs of the Endangered Species Act. At this point specific guidelines for the water quality for fish habitat in the Coho Creek have not been defined by DOE, but it can be assumed that stream dissolved oxygen concentration, nutrient concentration and temperature will be important.

The Quil Ceda Village environmental staff are active in improving fish habitat in the Coho Creek, and interested in processes that can further enhance the quality of the Class A treated water from the MBR WWTF. These include wetlands, aerated flow zones, and infiltration trenches before entering the stream. Wetlands have been used after WWTFs for further polishing, and to provide an environmental habitat for other wildlife and aesthetics.

Other compounds, found at very low concentrations, below 1 ug/L and termed micropollutants, are not presently regulated but may be of importance when considering fish habitat and reproduction. The compounds have been referred to as emerging pollutants of concern (EPOC) and consist of hundreds of compounds originating from pharmaceutical use and personal care products (PCPs). Pharmaceuticals include aspirin, ibuprofen, steroids, heart medications, antibiotics, and other prescription medicines. PCPs include compounds from fragrances, soaps, detergents, cosmetics, shampoos, and fabric softeners.

Many of these compounds are found to be endocrine disrupting compounds (EDCs) due to their ability to alter sexual characteristics of wildlife at trace levels. EDC are likely responsible for many reported cases of unusual sexual characteristics of wildlife. Examples include a report by the United States Geological Survey (USGS) that found fish in many streams to have atypical ratios of male and female sex hormones, studies cited by the United States Environmental Protection Agency (USEPA) showing disrupted endocrine function in "snails, oysters, fish, alligators and other reptiles, and birds, such as gulls and eagles", and a study from England where researchers found that male trout kept in cages near WWTF outfalls were developing eggs on their testes and had increased levels of the protein that is responsible for egg production (vitellogenin). Follow-up laboratory studies showed that synthetic forms of estrogen (EE2) could increase vitellogenin production in fish at levels as low as 1-10 ng/L, with positive responses seen down to the 0.1-0.5 ng/L level.

There are an enormous number of synthetic, organic compounds that potentially have the ability to disrupt the endocrine system, acting either as agonists (mimic hormones – bind to receptors) or antagonists (disruptors – prevent binding). Although many compounds have already been identified to be EDC, it is not known how many more EDC will be discovered. As the sophistication of analytical tools increases, and researchers search for previously unidentified trace contaminants, the list continues to grow. Studies looking at potential EDC have included: 82 organic wastewater contaminants found in 80% of streams sampled nationally pharmaceuticals and bisphenol A in surface water in New Orleans, and 62 organic contaminants including caffeine and pesticides in surface water in Iowa.

The natural and synthetic forms of human estrogens have been identified as being the major contributors to the total EDC activity of WWTF effluents. Formulas and chemical structure of the natural estrogens estrone (E1), 17β-estradiol (E2) and estriol (E3) and the synthetic estrogen 17α-ethinylestradiol are presented in Table 1. Most of the estrogenic activity of the WWTF effluent has been found in three studies to be due to E1, E2 and EE2.

The estrogens do not contribute equally to estrogenic activity based both on their varying concentrations in WWTF effluents together with their respective potencies, reported estrogenic activities are presented in Table 2. The low biological activity of E3 appears to make it less of an environmental concern in comparison to the other estrogens.

We have found in our research at the University of Washington that these estrogenic compounds are removed in wastewater treatment processes, but the mechanisms and conditions that control their level or removal are not well known. Bacteria responsible for nitrification in WWTFs

appear to have a great potential for estrogen compound degradation by cometabolic degradation and is currently under study. Other work suggests that systems with long solids retention times, like the MBR process, result in greater removal of estrogenic activity.

Table 1 name, formula and structure of estrogen compounds

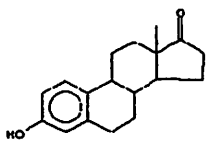
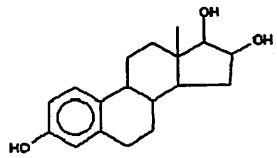
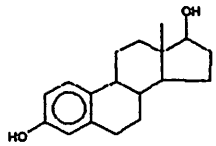
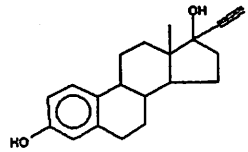
Name and formula	*Chemical Structure*		
E1 Estrone $C_{18}H_{22}O_2$		E3 Estriol $C_{18}H_{24}O_3$	
E2 17β-estradiol $C_{18}H_{24}O_2$		EE2 17α-ethinylestradiol $C_{20}H_{24}O_2$	

Table 2 reported estrogenic activity of estrogens in 17β-estradiol (E2) equivalents

reported estrogenic activity of estrogens as E2 equivalents	
E2	1
E1	0.5, 0.21
E3	0.005, 0.04, 0.0013
EE2	1, 2

Project Objectives

The major aim of this project is to develop and evaluate additional processes that can enhance the water quality in the MBR WWTF effluent before discharge in a managed fashion to the Coho Creek to support fish habitat and reproduction. Specific water quality goals will be identified. Alternative processes to follow the WWTF will be considered and evaluated. The system will include created wetlands; both submerged and surface flows. A pilot wetland system will be designed and a sampling and analytical program will be developed. The pilot system will have three flow paths to allow testing of polishing system design and process variables. The evaluation of the effluent polishing alternatives and the development of pilot effluent polishing system designs and testing program will be completed in Phase I.

Following the installation of the pilot polishing systems a long term testing program will be undertaken to evaluate the fate of nitrogen compounds and key endocrine disruptor compounds. This will be done in Phase II. Test work will follow key constituents in the current WWTF and through the polishing system and infiltration system prior to potential creek discharge.

The Phase I effort is describe here and the Phase II effort will be in a separate subsequent proposal.

Phase I Approach
A series of tasks are identified and briefly defined. For Phase I, the development of process alternatives and testing designs will be done by Professor H. David Stensel. He will be assisted by one quarter of a Master's student support for investigating related work through literature and internet surveys. Information from conference proceedings will also be obtained

Task 1. Identify key water quality parameters for polishing goals and to assess the performance of the pilot system.
We will work with the Quil Ceda Village staff to assess the water quality needs for Coho Creek and obtain literature information on stream augmentation applications and water quality. Nutrients are expected to be of concern, including ammonia and oxidized nitrogen. Phosphorus may also be of concern if there is sufficient sunlight and water detention time to allow for significant algae and plant growth from nutrient enrichment. If storage ponds are used there may be concern for algae growth and its effect on water quality in discharge to the stream. If the system's discharge has low nitrogen and high phosphorus, it may promote the growth of blue-green algae with associated nuisance problems including odors. We expect that dissolved oxygen and temperature will be of importance. It is possible that subsurface infiltration zones may have an effect on the discharge temperature.

We also propose evaluating the fate of selected endocrine disruptor compounds, especially the E1, E2, and EE2 compounds described above. One or two additional pharmaceutical compounds may also be selected within the capability of our laboratory instrumentation. We expect to have new instrumentation for a micropollutant research lab pending the receipt of a major grant under consideration.

Task 2. Identify and evaluate processes for polishing the WWTF Class A effluent.
A background search will be completed to identify wetland designs and other processes that can be considered prior to discharge of the reclaimed water into the Coho Creek. The background sources will include literature from refereed journals, trade journals, internet information and conference proceedings. The background information will focus on polishing processes used prior to stream augmentation and will identify specific design elements of these processes, including subsurface and surface wetlands. The design and performance information gained from this effort will provide the basis for the pilot wetland system design. The literature will also include information on the fate of EDC compounds in WWTF effluent through disinfection and wetland systems, where available.

Task 3. Design post treatment polishing pilot wetland system.
Based on the water quality parameters of interest form Task 1 and the information gained in Task 2, a polishing pilot wetland system will be design. This design may consider parallel systems for testing and will be done with review and input from the Quil Ceda Village staff, and with concern for construction schedules and budget limitations. The pilot system including a pond will have three flow paths each in the 12 gallons per minute (gpm) range for a pilot system capacity of 50,000 GPD.-The design and analytical program will be submitted before the end of

June 2007. Before the final submittal alternative designs will be reviewed with staff during May 2007. The designs will include system dimensions, a general layout, hydraulic consideration, sampling points, and operational issues.

Periodic memos will be issued for critical project steps and a final report will be issued that will include the background information, design alternatives and design basis, a testing program and a sampling and analytical program.

Schedule and Deliverables for Phase I

The schedule and deliverables from the listed tasks are given in the following table. The schedule is based on the project starting on December 15, 2006 and being completed by June 30, 2007.

Table 3. Schedule and Deliverables (Project start date is December 15, 2006)

Task	Completion Date	Deliverable
1. Identify water parameters	January 31, 2007	Memo with summary
2. Literature search on post treatment designs	April 1, 2007	Literature summary
3. Pilot system design alternatives review	May 31, 2007	Design alternatives summary memo with drawings
4. Pilot system design and sampling/analytical program	June 30, 2007	Design memo with drawing/sketches' Memo with QA/QC

Phase I Budget

The budget is for about a 6 month project period. One quarter of graduate student funding, including the student stipend and student tuition to assist on background information collection is included. Four weeks of salary support are included for Professor Stensel's time to assess background information, develop polishing treatment alternatives, review water characteristics and process alternatives with Quil Ceda Village staff, and to develop the final system design recommendations. The University of Washington standard overhead rate of 55.5% is applied to all costs with the exception of the student tuition cost. The total cost for the Phase I budget is $39,332 and it is submitted as a fixed price contract with an invoice to the Village before Dec 31, 2006.

Evaluation of Water Reclamation for the Tulalip Tribes Quil Ceda Village
Phase II
Professor H. David Stensel
University of Washington
October 23, 2006

Background

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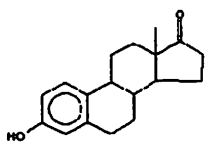
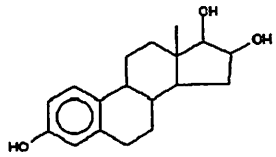
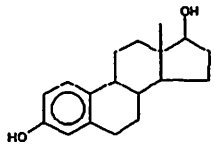
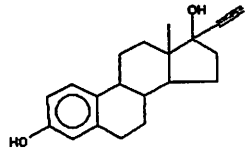
E1 Estrone $C_{18}H_{22}O_2$		E3 Estriol $C_{18}H_{24}O_3$	
E2 17β-estradiol $C_{18}H_{24}O_2$		EE2 17α-ethinylestradiol $C_{20}H_{24}O_2$	

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Project Objectives

The major aim of this project is to develop and evaluate additional processes that can enhance the water quality in the MBR WWTF effluent before discharge in a managed fashion to the Coho Creek to support fish habitat and reproduction. Specific water quality goals will be identified. Alternative processes to follow the WWTF will be considered and evaluated. The system will include created wetlands; both submerged and surface flows. A pilot wetland system will be designed and a sampling and analytical program will be developed. The pilot system will have three flow paths to allow testing of polishing system design and process variables. The evaluation of the effluent polishing alternatives and development of the pilot effluent polishing system designs and testing program will be completed in Phase I.

Following the installation of the pilot polishing systems a long term testing program will be undertaken in Phase II to evaluate the fate of nitrogen compounds and key endocrine disruptor compounds. Test work will follow key constituents in the current WWTF and through the polishing system and infiltration system prior to potential creek discharge. The proposed Phase II effort is described here.

Phase II Approach

The work in Phase II will be done primarily by a Master's degree graduate student in the Civil and Environmental Engineering Department at the University of Washington under the direction of Professor H. David Stensel. The student will first develop an understanding and proficiency in the necessary analytical methods and the carry out the sampling and analytical program laid out in Phase I. The test program is expected to occur over a 12 month period from December 2007 to December 2008. A series of tasks are identified and briefly defined.

Task 1. Develop and demonstrate analytical methods for selected EDC compounds

The student will first develop skills and quality assurance and quality control (QA/QC) for the necessary analyses. Analytical methods and precision must be demonstrated for low level concentrations of chemical oxygen demand (COD), ammonia, nitrite, nitrate, and phosphorus Analytical methods for estrogenic compounds are already in place at the environmental engineering lab and the Master's student will work with a Ph.D. student to develop the analytical skill and precision needed. The estrogen analytical methods are attached in Appendix A. We anticipate that additional analytical techniques may be applied using LC-MS for 2-3 other pharmaceutical chemicals. The Yeast Estrogen Screening (YES) method, described in Appendix B, will also be used to assess potential endocrine disruptor activity of products from estrogen degradation. This analytical background effort is expected to take 4 months.

Task 2. Evaluate the fate of selected compounds in the membrane bioreactor reactor (MBR) system, disinfection process and pilot polishing systems and infiltration trench on the removal of key constituents.

This task represents the key aim of the Phase II effort. The goal is determine how the MBR system and different polishing processes affect the removal of nutrients, organic compounds, and endocrine disruptor compounds (EDCs). Nitrogen species, endocrine disruptor compounds, phosphorus, and organic carbon concentrations will be followed through different process steps, which will be defined in the sampling and analytical program in Phase I. The student will obtain samples from the site and perform test work in the University of Washington Environmental Engineering labs. Alternative operating modes may be considered in the WWTF and the student will investigate the removal of EDCs by the disinfection process and methods to improve the EDC removal there. A Yeast Extraction Screening (YES) procedure will be used (described in appendix) to determine if the parent EDC degradation product has estrogenic activity. The removal of the EDCs and other compounds will be compared for the parallel polishing systems. It is assumed that composite sampling equipment will be supplied by the site.

This activity will be done over a course of year to assess the effect of operating temperatures on the performance of the MBR and polishing systems for removal of the selected compounds.

Task 3. Provide final report

This task will follow the final writing of the M.S. thesis and will describe the fate of the selected constituents and how the processes affect their removal. Impacts on improving fish habitat and the receiving stream quality will be assessed.

Schedule and Deliverables for Phase II

The schedule and deliverables from the listed tasks are given in Table 3. The schedule is based on the project starting on September 15, 2007 and being completed by March 15, 2009.

Phase II Budget

The budget is for an 18 month project period. A Master's candidate graduate student will be funded over this time and the budget includes the student stipend and student tuition. The supply budget for analytical chemicals is $14,000. Two weeks of salary are included for the project management and direction by Professor H. David Stensel. The University of Washington standard overhead rate of 55.5% is applied to all costs with the exception of the student tuition cost. The total cost for the Phase II budget is $103,229 and it is submitted as research contract. The project invoices to the Village will be done on a quarterly basis.

Table 3. Schedule and Deliverables (Project start date is September 15, 2007)

Task	Completion Date	Deliverable
1. Establish analytical methods and QA/QC	December 31, 2007	Memo showing standard curves and analytical precision
2. Evaluate removal of selected constituents through MBR disinfection and polishing system	December 15, 2008	Quarterly reports with data
3. Complete final report	April 1, 2009	Final report

Appendix A
Estrogen Analytical Methods

Due to the difficult nature of quantifying estrogen concentrations at environmentally relevant concentrations down to 1 ng/L analytical method development has represented a significant amount of preliminary laboratory work. The following work has been accomplished toward method development.

LC-MS-MS

The following method has been developed in collaboration with Tom Kalhorn at the University of Washington Medicinal Chemistry Mass Spec Laboratory for analysis of EE2 with LOQ to 100 ng/L. The same method will be used for E1, E2 and E3 following identification of their daughter ions in single ion monitoring (SIM) mode.

Prior to use all glassware is washed, air-dried, rinsed with methanol (MeOH) and again air-dried (Desbrow et al. 1998). A standard for calibration of the LC-MS-MS was made by dissolving 100 mg/L EE2 in MeOH, lower concentrations were then made by 1:10 serial dilutions. 50 µL sample volume was analyzed with a Shimadzu HPLC and Micromass Quattro II tandem quadrupole mass spec (MS-MS). A Gemini 5 C_{18} 110A, 150x2mm column was used for further separation. The mobile phase was ACN and NH_4OH at a flowrate of 0.3 ml/min with the following gradient: 70:30 ACN:NH_4OH (0-2 min), 100 ACN (2-3 min), 70:30 ACN:NH_4OH (3-5 min). The MS is operated in electrospray ionization, negative ion mode. The molecular weight of EE2 is originally 296 g/mol, following ionization to the $(M-H)^-$ at m/z 295 (1^{st} MS step), the molecule is then fragmented into daughter ions of 199 and 145 m/z (2^{nd} MS step) using Ar as the collision gas.

Running standards with this method have reliably produced LOQ to 100 ng/L. A trial assay with *N. europaea* employing the same methods as described in the preliminary experiments section was ran with sampling of estrogen concentrations spiked from 100 ng/L to 10,000 ng/L to evaluate sample recovery. Sample recovery was poor and was determined to most likely be due to sorption to the 0.2 µm HPLC PVDF syringe filter. The next trial assay will be conducted using centrifugation to separate biomass from sample eliminating the filtration step.

Solid Phase Extraction (SPE)

When quantification is desired at concentrations less than 100 ng/L the sample must be concentrated by solid phase extraction (SPE) 100Xs to reach the LC-MS-MS level of quantification. The following C_{18} extraction method is based on the standard operating procedure developed by King County Environmental Laboratory, which was based on (Ternes et al. 1999a).

JT Baker disposable polypropylene columns prepacked with 500 mg C_{18} were purchased from Alltech. An Alltech vacuum manifold was used to control flow through the columns throughout the SPE procedure. Columns were prepared with MeOH and H_2O by applying 5 mL MeOH to the column with a 1-2" Hg vacuum until a few drops pass through the sorbent. The valve is then closed allowing MeOH to soak the sorbent bed for 1 minute. Then a 1-2" Hg

vacuum is applied drawing the solvent down to 2 mm above the top surface of the column sorbent. Liquid volumes are always brought down to this level, never allowing the sorbent bed to go dry. 10 mL of H_20 is then applied to the column with a 2" Hg vacuum. Following column preparation, 50 mL of sample is applied to the column with a 2" Hg vacuum. The column is then washed by adding successively 5 mL H_2O, 3 mL 40:60 MeOH:H_2O, and 3 mL 20:80 acetone (ACE):H_2O, all with a 2" Hg vacuum. Once the ACE:H_2O has been washed through the column the vacuum is increased to 7" of Hg for 20 minutes to dry the sorbent. The sample is then desorbed by applying 3 mL MeOH to the column. The vacuum is increased to 8-9" Hg long enough to let a few drops pass through. The valve is then closed allowing the sorbent to soak for 1 minute. The vacuum is then reduced to 1-2" Hg to draw the sample volume down halfway. The sorbent is then left to soak for another minute. Finally, a vacuum of 1-2" Hg is applied to draw the remaining sample through until all dripping has entirely stopped. Samples are then evaporated under a gentle stream of nitrogen gas and brought to a final volume of 50 µL in 65:35 MeOH:H_2O.

Preliminary work has been done evaluating the method for different sample volumes. Sample matrices are also being evaluated, current work has been with AOB medium, protocol modifications might be necessary to account for a more complex sample matrix (e.g. wastewater). Sample recovery at each step of the SPE was evaluated with the LC-MS-MS. A recovery of 70% in the final concentrated sample was achieved when starting sample concentrations were 100 ng/L, concentrating 100x to 10 µg/L. With starting sample concentrations of 1 ng/L the LC-MS-MS sample injection volume had to be reduced to 25 µ L from 50 µ L to reduce the width of sample peaks for their identification and quantification. The smaller injection volume reduced the mass of sample on the column and hence also lowered sensitivity. Quantification was lost below 3 µg/L (needed to be down to 0.1 µg/L), although detection was still possible. The derivatization method described in Anari et al. (2002) is being evaluated to increase sensitivity and alleviate this problem.

Appendix B
Yeast Estrogen Screen (YES)

Permission to use the YES was obtained from Dr. Sumpter of Brunel University who developed the YES at Glaxo, the yeast was kindly provided by Dr. Love at Virginia Tech. The method used is as described by Routledge and Sumpter (1996) with some modifications as described in Beresford et al. (2000). The YES employs genetically modified yeast, *Saccharomyces cervevisae,* which contains the gene for the human estrogen receptor (hER) in its chromosome. The hER is linked to an expression plasmid with a *lac-Z* reporter gene, which encodes the enzyme β-galactosidase. When a ligand binds to the hER, it ultimately results in β-galactosidase being secreted into the assay medium which contains chlorophenol red-D-galactopyranoside (CPRG). The β-galactosidase causes the CPRG to change from yellow to red, the CPRG color change can be quantified by absorbance at 544 nm, with results reported as E2 equivalents of estrogenic activity. Plate readings are corrected for turbidity with the following formula:

chemical reading at 544nm – (chemical reading at 620nm – solvent blank reading at 620nm)

Trial assays were conducted with estrogen samples in either MeOH or in AOB medium with both 5 and 10 μL sample volumes. Assays are carried out in 96-well microtiter plates with a final volume in each well of 200 μL. Samples in MeOH were evaporated in the well plates, followed by addition of 200 μL seeded YES assay medium. Samples in AOB medium were added directly to the seeded YES assay medium in the well plates such that the final volume was 200 μL. Plates were sealed with sealing film and plate edges with tape. Plates were shaken daily on an orbital shaker for 5 minutes. Assays were incubated for 3 days at 30°C and then at room temperature (20°C) for an additional 2 to 11 days for optimum color development, with readings taken at 5, 8 and 11 days. Samples were run in duplicate or triplicate, each plate contained repetitions of an E2 standard curve and solvent in yeast blanks. Dose response curve results obtained from two trial assays are presented in Figure A1 and Figure A2.

The level of detection (LOD) for E2 concentrations in the well plate is 3 ng/L. The sample is diluted 20-40 fold with the YES assay medium, if the sample is delivered as 10 μL of evaporated sample, the sample LOD should be 60 ng/L (Routledge and Sumpter 1996). For LOD lower than 60 ng/L the SPE method described above can be used. The LOD for estrogens other than E2 will depend on their estrogenic activity (e.g. see Table 2). Adding the sample as a liquid directly to the YES assay medium reduces the yeast cell density over the evaporation method (same final volume of 200 μL), which can potentially reduce the sensitivity of the assay, but sample delivery in a liquid that is not evaporated can also increase the sensitivity (Beresford et al. 2000).

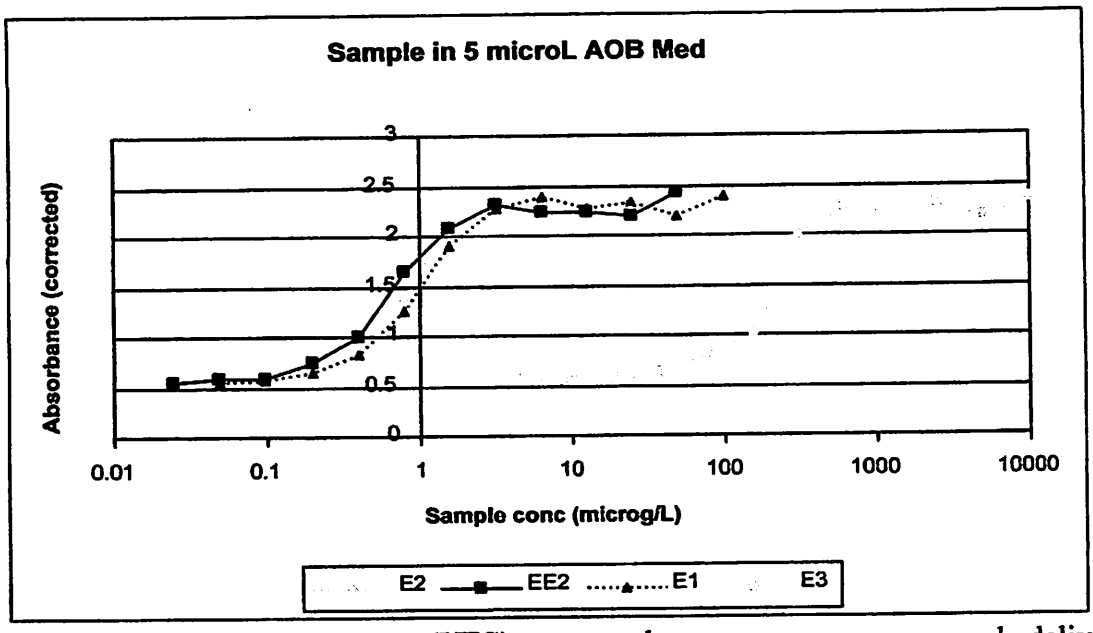

Figure A1 yeast estrogen screen (YES) estrogen dose response curves, sample delivery to assay in ammonia oxidizing bacteria (AOB) medium

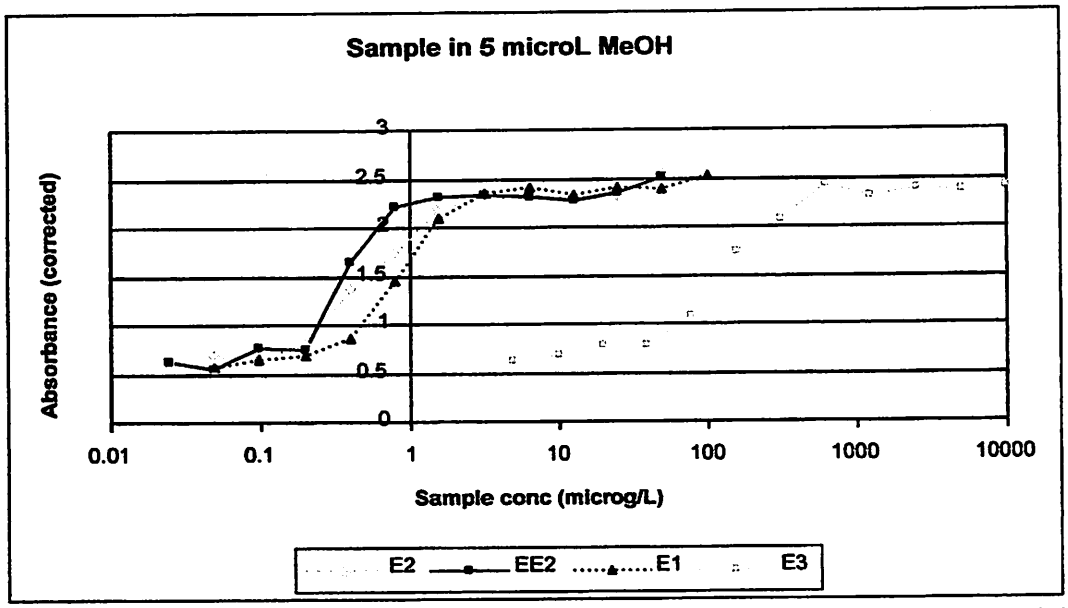

Figure A2 yeast estrogen screen (YES) estrogen dose response curves, sample delivery to assay in evaporated methanol (MeOH)

Appendix B
Facilities, Equipment and Other Resources

The Environmental Engineering laboratory, consisting of over 8000 ft^2, is well equipped to carry out the basic bench scale reactor studies in batch and continuous fed reactors. The lab contains 3 walk-in temperature control rooms for test reactors, as well as a reach in temperature chamber and 3 temperature-controlled shakers. Necessary equipment for bioreactor studies is available, including peristaltic pumps, mixers, temperature controllers, syringe pumps, and chemostats. We have maintained pure culture reactors p in previous studies, and techniques and resources for providing a sterile feed and aeration source are available. Funds are requested for a dissolved oxygen probe and controller. Chemical analysis such as COD, pH, alkalinity, ammonia, nitrate, nitrate, and gas methane are performed in many of our studies. The analytical instrumentation available in this lab includes GC-FID, GC-ECD, HPLC, and Ion Chromatography. Our estrogen analysis methods have been developed through arrangements with the University of Washington Medicinal Chemistry Mass Spectrometry Center for the use of LC-MS-MS instrumentation. In summer of 2007 we will have our own and trace organic analysis laboratory, equipped with a LC-MS-MS. The project team has also worked with the King County Environmental laboratory with estrogen measurements methods. They are able to provide consultation and QA/QC comparisons. Work with molecular tools to follow the nitrifying populations is possible through equipment in the Environmental Engineering program microbial ecology lab headed by Dr. Stahl. We have worked in this lab and with highly qualified post-doctoral researchers applying molecular biology tools such as PCR, cloning, FISH with a confocal laser microscope, RFLP and T-RFLP.

In short, the facilities and equipment are available for this study. As described in the Technical Description most of the analytical methods are underway and methods are available to detect estrogen compounds at the ng/L level.